# The centrifugal partition reactor, a novel intensified continuous reactor for liquid–liquid enzymatic reactions

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A B S T R A C T

Implementation of continuous processes for production of fine chemicals and pharmaceuticals is an efficient way for process intensification. This study aims at demonstrating the potential of a Centrifugal Partition Chromatography (CPC) apparatus as a novel type of intensified reactor (termed Centrifugal Partition Reactor, CPR) for biphasic (water-organic solvent) enzymatic reactions. The reaction of esterification of oleic acid with n-butanol catalyzed by the *Rhizomucor miehei* lipase was tested as the model reaction.

The influence of rotation speed, flow rate, enzyme and substrate concentrations on esterification reaction were studied. The CPR proved to be efficient to generate sufficient interfacial area (weakly dependent of the flow rate) and sufficient residence time (30 min) to achieve good conversion (85%). Also, increasing rotation speed of the CPR surprisingly decreased performances, probably due to very specific inner hydrodynamics. For a given configuration, the productivity of the CPR ($40.5\,g\,h^{-1}\,L^{-1}$) was found to be more favorable than the conventional batch process ($21.6\,g\,h^{-1}\,L^{-1}$). Steady state operation of the reactor at 22 °C, (i.e., constant conversion at the output, see Fig. 8), was reached after about 2 residence times and lasted for 24 h. After 24 h, the output conversion slowly decreased due to the low intrinsic stability of the enzyme at room temperature.

The promising results obtained in this study are a good incentive to promote the CPR as a competitive innovative technology for operating continuous two phase enzymatic reactions.

## 1. Introduction

Lipases (EC 3.1.1) catalyze hydrolysis, esterification, inter- and trans-esterification reactions in aqueous or non-aqueous media [1]. Lipase-catalyzed esterification reactions have gained growing interest during the last decades due to an increased use of organic esters in the biotechnology and chemical industry (food, detergents, plasticizer, lubricant, etc.) [1–2]. Furthermore, some studies have been published on enzymatic esterification with the aim to improve biofuel production [3–5]. The lipase esterification in two phase media (water-organic system) offers several advantages:

1. the thermodynamic equilibrium of the reaction is switched towards synthesis [4],
2. the solubility of non-polar substrates and products is high,
3. the enzyme is located in a favorable aqueous environment at the interface,
4. it is possible to directly use an aqueous enzyme solution, avoiding the need of immobilization onto a solid support,
5. the separation between catalyst and products is easy [6–7].

Therefore, lipase-catalyzed esterification in such systems may gain considerable industrial potential and there is a need to propose intensification of this process to increase productivity and to ensure economic viability. In this case intensification is achieved by implementing continuous operation of a reactor with efficient mixing, high interfacial area and robustness of operation. Recently, some attempts were done to perform this kind of reaction using different novel technologies of intensified continuous reactors. Elgue et al. [8] successfully tested the Corning type and Chart type reac-

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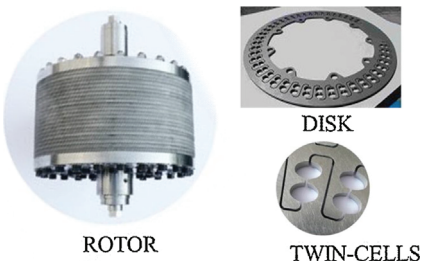


**Fig. 1.** The rotor and an engraved disk composed of twin-cells connected by ducts. The pictures were from Armen Instrument catalog [15].

tors. Such continuous reactors have the advantage to promote mass transfer and to generate high interfacial area. However, their performances depend on flow rates and maximizing them implies an increase in flow-rates, which correlatively shortens the residence time. This could be a problem for enzyme operating at liquid–liquid interface where global kinetics is highly dependent upon interfacial area. In addition, in this type of reactor, the aqueous enzymatic phase is continuously fed simultaneously with the substrate containing phase. Thus, the enzymatic aqueous phase needs to be separated at the output of the reactor in order to be recycled. This requires an inconvenient operation of decantation which is not always easy to operate in the continuous mode. So an intensified reactor able to "immobilize" the catalytic aqueous phase and where mass transfer performances would be weakly dependent upon flow rates, would be very useful to develop efficient intensified enzymatic processes.

Such a concept is investigated here through the use of an existing apparatus, diverted from its original application, the Centrifugal Partition Chromatograph (CPC, also called Hydrostatic CounterCurrent Chromatography). This system is originally and conventionally used for separation purposes (support-free liquid chromatography or extraction). Basically, a CPC apparatus consists in a series of cells engraved on a disk and connected in cascade by ducts. Disks are stacked to form a column called "rotor" (Fig. 1). This latter is rotated and this assembly is then submitted to a constant centrifugal field (several hundreds of g) [9]. This centrifugal field enables maintaining one of the liquid phases inside the cells (the stationary phase) while the other one (the mobile phase) percolates this stationary phase in each cell as a jet. Depending on the physico-chemical properties of the two phase system, the jet may disintegrate as a film along the cell wall or as very tiny droplets [10]. It then coalesces to leave the cell and flows to the next cell via a tiny duct. When this apparatus is operated in the so-called "ascending" mode, the heaviest phase (here the aqueous phase containing the enzyme) is retained in the column as the stationary phase. The understanding of the CPC functioning, especially the influence of rotational speed and mobile phase flow-rate, upon the liquid–liquid dispersion and the value of the stationary phase hold-up, is complex and its exhaustive description is out of the scope of this paper. The reader is advised to refer to some dedicated papers such as the book of Foucault [9] or the paper of Marchal et al. [10].

In this work, it is proposed to operate this system as a two liquid phase reactor (termed Centrifugal Partition Reactor, CPR) as already proposed as a continuous reactor for catalysis [11–12] and as a chromatographic reactor for enzymatic reactions [13–14]. Indeed, in the CPC apparatus, one of the phases, containing the enzyme catalyst, can be maintained in the reactor while the other one, carrying substrates and products, is continuously fed and extracted from the system. Basically, it can be said that the whole cascade of liquid–liquid contacts in each cell (droplet (or film) generation then coalescence) is equivalent to a continuous reactor with an immobilized catalytic phase. Because the very high number of contact cells (usually in the range 200–2000), the back mixing is reduced and the CPR can be easily assimilated to a continuous plug flow reactor [12].

Fig. 1 presents pictures of the elements of a CPR: the rotor and one of the engraved disks composed of twin-cells connected by ducts.

In the present case, the main advantages of this type of device lies in its ability to immobilize a liquid phase containing the enzyme while generating a large interfacial area with the organic phase containing the substrates. In this sense, the system behaves similarly to a conventional fixed bed reactor of porous particles where enzyme is immobilized onto the inner surface of particles. Note that when efficient immobilized enzymes are easily available the fixed bed reactor is undoubtedly a very good reactor technology. It is important to note that it has been shown that in CPC the interfacial area is only slightly dependent upon the flow rate of the mobile phase [9] and therefore, in such a system, a suitable residence time can be achieved by adapting the mobile phase flow rate and makes it possible to operate moderately fast enzymatic reactions. In this work, the potential of CPC apparatus to perform two phase enzymatic esterification was investigated using esterification of oleic acid with *n*-butanol, catalyzed by lipase from *Rhizomucor miehei*, as a model reaction. Heptane was used as the organic phase containing oleic acid and *n*-butanol substrates and the immobilized aqueous phase consisted of a solution of *R. miehei* lipase in a phosphate buffer (pH 5.6). The aim of this work was to prove the feasibility of operating this reaction in such a continuous liquid–liquid centrifugal reactor. The main parameters usually influencing enzymatic esterification (oleic acid/*n*-butanol molar ratio, lipase concentration, substrate concentrations) and operating parameters of the CPR (rotation speed and flow rate) were evaluated. A comparison between this novel CPR technology and conventional batch agitated vessel reactor is also reported.

## 2. Methods

### 2.1. Materials

The *R. miehei* lipase, produced in *Aspergillus oryzae* was purchased from Sigma–Aldrich Chemie (Saint-Quentin Fallavier, France). Lipase activity (918 AU $mL^{-1}$) was spectrophotometrically determined by following the hydrolysis of *p*-nitrophenyl butyrate (*p*NPB) at 405 nm. For this purpose, an aliquot of the enzyme solution (20 μL) is added to a reaction mixture composed by 175 μL of 100 mM of phosphate buffer (pH 7.2) and 5 μL of *p*NPB (40 mM in 2Methyl2Butanol). The mixture is incubated at 25 °C for 10 min. One unit of lipase activity (U) is defined as the amount of lipase required to release 1 μmol of *p*NPB per minute, under the specified conditions (25 °C and pH 7.2).

Oleic acid (purity 95%) was obtained from the same provider. All other chemicals were of analytical grade (heptane, *n*-butanol, methanol and ethanol) and purchased from Fisher Scientific (Illkirch, France). The standard for HPLC analysis of *n*-butyl ester (purity > 95%) was obtained from Combi-Blocks, Inc. (San Diego, CA, USA).

### 2.2. Experiments and conditions

#### 2.2.1. CPR experiments

The experiments were performed in a CPC-250-F apparatus, manufactured by Armen Instrument (Saint-Avé, France). The column consisted of 21 disks stacked as a rotor. Each disk is composed of 90 twin-cells (100 μL volume for each cell) linked in cascade by tiny ducts. From tracer experiments, the actual column volume (cell and ducts) was estimated at 200 mL. The liquid–liquid system consisted of an organic phase composed of *n*-butanol dissolved in

heptane and of a phosphate buffer aqueous phase at pH 5.6 containing the enzyme. The system was physico-chemically equilibrated before use into the CPR. This means that both phases were shaken three times a few seconds in a glass flask at room temperature (22 °C) and let to settle for 2 h.

For easier interpretation of results, all experiments were performed at the same value of the stationary phase hold-up. The chosen value corresponds to the one obtained after hydrodynamic equilibration at the highest flow rate and lowest rotation speed of our experimental set up (15 mL/min and 800 rpm respectively). As a consequence, when the flow rate is decreased or the acceleration increased, no more stationary phase is displaced and the hold-up is maintained constant.

A typical experiment was done as follows: first the CPR is totally filled with the aqueous phase at the appropriate concentration of enzyme (from 0.5 g $L^{-1}$ to 15 g $L^{-1}$). Then, while rotating, the mobile phase without oleic acid is pumped up to hydrodynamic equilibrium and the liquid hold-up is evaluated by measuring the volume of the expelled aqueous phase (in our case hold-up is 70 %, meaning that 30% of the aqueous phase is expelled from the system). Then the mobile phase pump is switched to pump an equilibrated organic phase that now contains the oleic acid substrate (usually at 0.032 mol $L^{-1}$).

Steady-state operation (meaning constant oleic acid concentration at the output) was usually reached after two times the residence time at the given operating conditions. The residence time is conventionally defined as the volume of mobile phase in the reactor (here 30% of the total column volume) divided by the mobile phase flow rate. Note that all experiments were conducted at controlled room temperature (22 °C).

At the reactor output, the mobile organic phase with reacted oleic acid was sampled at regular time intervals. The decrease of oleic acid concentration was measured by a titrimetric method as described in the analytical methods paragraph. The concentration of the product, *n*-butyl oleate, was evaluated by HPLC analysis (see also analytical methods paragraph in Section 2.3).

#### 2.2.2. Batch experiments

As a comparison, esterification reactions were also conducted in a conventional batch mode, using a 200 mL agitated glass reactor, equipped with a four- blade turbine impeller. The same liquid–liquid system as for CPR experiments was used. For these experiments reaction mixture consisted of a 70/30 v/v (aqueous/heptane) two phase mixture. In a typical experiment the aqueous phase (130 mL) contained the dissolved enzyme at 3 g $L^{-1}$ (917 AU $mL^{-1}$ of enzyme) in a phosphate buffer solution 0.1 M (pH 5.6). The organic phase (70 mL) contained 0.6 g of oleic acid (0.032 mol $L^{-1}$) and 0.5 g of *n*-butanol (0.096 mol $L^{-1}$) dissolved in heptane.

The reaction mixture was agitated at controlled room temperature (22 °C) for 240 min at different impeller rotation speed (800, 1200 or 1600 rpm). Samples were periodically withdrawn from the organic phase. Sampling of the organic phase was done after agitation was stopped and system rapidly decanted. The samples were analyzed by the titrimetric method (see Section 2.3).

### 2.3. Analytical methods

Two methods were used to assess the conversion of the reaction. The first one, termed the titrimetric method, is very simple but only allowed to estimate the oleic acid conversion. In this method, each sample (2 mL) was dissolved in 10 mL of ethanol and supplemented with a few drops of phenolphthalein (1% alcoholic solution) as an indicator and titrated for the residual oleic acid content, using a 0.01 M KOH solution (in ethanol). The substrate conversion was calculated comparing the total acid concentration in the sample after reaction with the one found before the reaction.

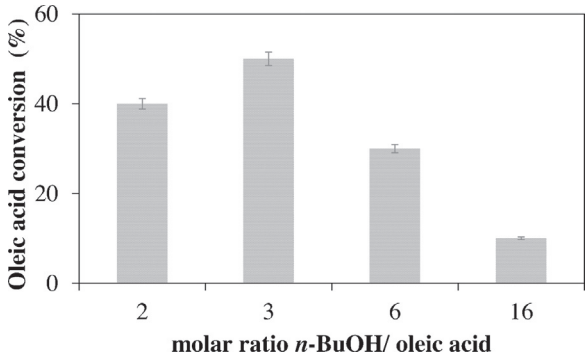


**Fig. 2.** Effect of *n*-butanol to oleic acid molar ratio (2, 3, 6 and 16) upon oleic acid conversion in the CPR. The feed oleic acid concentration and enzyme concentration were 0.032 mol $L^{-1}$ and 3 g $L^{-1}$, respectively. The rotation speed was 1200 rpm and mobile phase flow rate was 10 mL $min^{-1}$. CPR is operated at controlled room temperature (22 °C). The reported conversion values are the average of triplicate experiments.

The second method, using an HPLC system, enabled the concentration of oleic acid and also of *n*-butyl oleate in the organic phase to be measured using a Dionex HPLC, equipped of a $C_{18}$ column (Hypersil Gold, 150 × 2, 1 mm 3 μm; Thermo Fisher), a temperature controlled column compartment, a UV detector and a Chromeleon Chromatography Data System. The mobile phase was methanol with an isocratic flow rate of 0.2 mL $min^{-1}$. An HPLC-Corona Charged Aerosol Detector (Corona CAD), from Thermo Scientific (Villebon-sur-Yvette, France), was placed in-line after the UV–vis variable wavelength detector. Nitrogen gas was used as the nebulizer gas for the Corona CAD at a pressure of 35 psi. For all samples collected at the output of CPR, the organic solution was evaporated to eliminate the solvent and the concentrated mixture was dissolved with the HPLC mobile phase. To evaluate oleic and ester concentrations after enzymatic reaction, analytical standards were used. A solution of 32 mmol $L^{-1}$ of each standard was prepared and then diluted with the HPLC mobile phase to obtain various concentrations, 16, 8, 4 and 2 mmol $L^{-1}$.

The esterification conversion calculated by both methods (titrimetric and HPLC analysis) were found to be in good agreement.

## 3. Results and discussion

### 3.1. Alcohol to acid molar ratio effect on reaction performance in CPR

It is known that the alcohol to acid molar ratio influences the esterification kinetics [16–18], alcohol being a substrate and also a competitive inhibitor of the reaction. Thus, different *n*-butanol to oleic acid molar ratios (2, 3, 6 and 16) were tested for the esterification on the CPR. This was done at 3 g $L^{-1}$ enzyme concentration and 0.032 mol $L^{-1}$ oleic acid concentration. The rotation speed was 1200 rpm and the flow-rate of the organic mobile phase was 10 mL $min^{-1}$, corresponding to a 8 min residence time value. As shown in Fig. 2, the oleic acid conversion increases with increasing molar ratio up to a maximum value of 50% for the ratio equal to 3. Further increase of this ratio drastically decreased the conversion (to 30% and to 10% at molar ratio of 6 and 16, respectively). Several works have already evidenced such inhibitory effects of high concentration of *n*- butanol for esterification reactions [8,17,18]. Such a decrease is in accordance with the mechanism of this type of reaction, known as Ping Pong Bi Bi mechanism, where alcohol is a competitive inhibitor [16,18]. Note that, for each molar ratio, a batch reaction was conducted up to thermodynamic equilibrium of

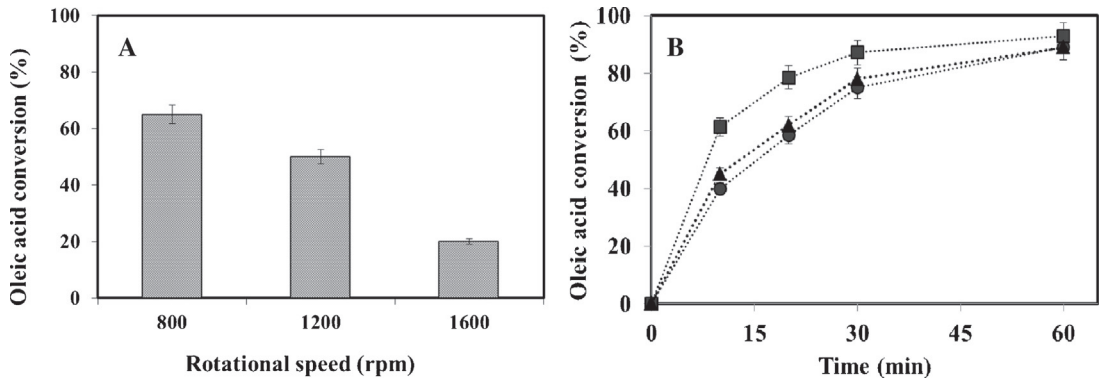


**Fig. 3.** (A) Effect of rotation speed on the oleic acid conversion in the CPR. *n*-BuOH/oleic acid = 3, *R. miehei* concentration = 3 g $L^{-1}$, mobile phase flow rate 10 mL $L^{-1}$ corresponding to 8 min residence time. (B) Effect of impeller rotation speed (●: 800 rpm, ▲: 1200 rpm, ■: 1600 rpm) in the batch reactor, at the same substrate molar ratio and enzyme concentration as in the CPR. CPR and batch reactor were operated at controlled room temperature (22 °C). The reported conversion values are the average of triplicate experiments.

the reaction in order to insure that the conversion in the CPR was actually only limited by kinetics and not by the thermodynamic equilibrium of the reaction.

As a conclusion, these first results have ascertained the feasibility of using the CPR as a continuous two phase reactor where the catalytic aqueous phase is immobilized, as it could have been done using a solid porous support.

### 3.2. *Effect of the rotation speed*

Interfacial area and mass transfer are key factors in two phase lipase-catalyzed reactions because such enzymes are known to act at the aqueous-organic interface [19]. Interfacial area and mass transfer are highly dependent on the hydrodynamics of the two phase system [8]. In the case of a biphasic tank reactor, increasing the impeller rotation speed increases interfacial area. This is shown by the results obtained in the batch reactor (Fig. 3B) where oleic acid conversion was observed to increase with impeller rotation speed. These results are in accordance with results previously presented in the literature for stirred stank reactors [20–21]. These authors have shown that increasing the rotation speed resulted in increased total interfacial area. This phenomenon is due to shear stress increase which causes the breakage of large oil droplets into smaller ones. Such phenomenon is for instance accounted for by the basic empirical model proposed by Calderbank [22].

In conventional utilization of the CPR, increase of rotation speed creates a higher acceleration field and, for systems like heptane–butanol–water, this usually leads to higher stationary phase hold-up, increased mobile phase dispersion and stationary phase mixing [9,23]. This has a positive effect on the interfacial area and on the overall interfacial mass transfer and is expected to improve kinetics [20,21].

Using the best operating conditions determined above (molar ratio of 3, 3 g $L^{-1}$ of lipase from *R. miehei*, 10 mL $min^{-1}$ flow rate), three different rotation speeds were tested. The conversion was 65% at 800 rpm, 50% at 1200 rpm and 21% at 1600 rpm (Fig. 3A). These results showed that the conversion surprisingly decreased when the rotation speed was increased.

Indeed, this behavior is directly related to our specific operational procedure (see Section 2) where the CPR is hydrodynamically equilibrated at the highest flow-rate and lower rotation speed. Equilibration at lower flow-rate, for systems like heptane–butanol–water, would yield higher stationary phase hold-up. But in our experimental procedure, where the hold-up is maintained constant, when increasing the acceleration field, the mobile phase volume in each CPR cell becomes larger than the "theoretical" volume at hydrodynamic equilibrium. So, it can be hypothesized that the excess volume forms a larger fraction of coalesced mobile phase at the cell outlet. This phenomenon reduces the active dispersed biphasic zone in the cell, so the interfacial area.

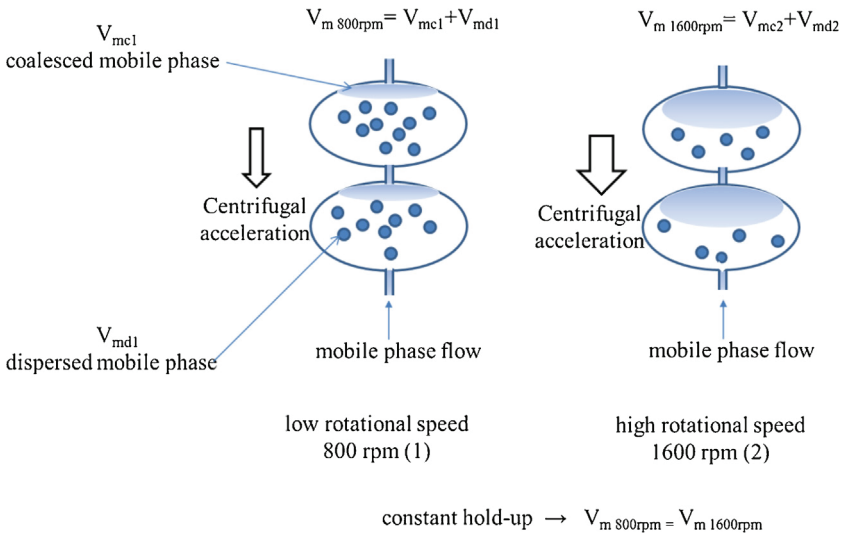


**Fig. 4.** Scheme of the probable hydrodynamics in the CPR when increasing the rotation speed at constant stationary phase hold-up.

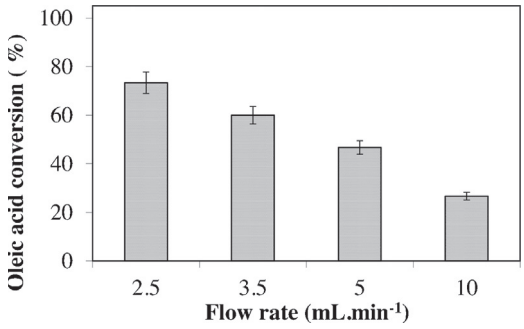


**Fig. 5.** Effect of mobile phase flow rate on the oleic acid conversion in the CPR. Operating conditions: *n*-BuOH/oleic acid = 3, *R. miehei* concentration = 0.5 g $L^{-1}$, rotation speed 800 rpm. CPR is operated at controlled room temperature (22 °C).

This hypothesis is schematically illustrated in Fig. 4. This result emphasizes the importance of the suitable choice of the set of values: flow-rate, rotation speed and desired hold-up.

Note that, similarly, a diminution of conversion at higher rotation speed was also observed for other type of centrifugal reactors [24]. In these reactors the volume of reactive dispersed phase in the centrifuge was shown to be a function of the rotation speed and decreases considerably at high rotation speed, thus decreasing the interfacial area.

### 3.3. Effect of mobile phase flow rate

In the case of so-called "dynamic mixing intensified reactors" (Corning reactor, Chart reactor...), increasing flow rate directly decreases the residence time but correlatively increases mass transfer and interfacial area [8]. This partially compensates the negative effect of the residence time reduction and this results in a weak influence of flow rate [8] upon conversion. In the CPR, influence of mobile phase flow-rate was studied in the range of 2.5–10 mL $min^{-1}$. Experiments were performed at a 0.5 g $L^{-1}$ lipase concentration with an alcohol to acid molar ratio of 3 and a rotation speed of 800 rpm. Note that these experiments were specifically carried out at low lipase concentration to prevent from reaching thermodynamic equilibrium of the reaction in the reactor, especially at high residence time (at low flow rates).

Fig. 5 clearly shows that oleic acid conversion is logically a decreasing function of mobile phase flow rate and as expected, the maximum conversion (73%) was obtained for the lowest flow rate of 2.5 mL $min^{-1}$. At 2.5 mL $min^{-1}$ the residence time in the CPR was around 30 min while at 10 mL $min^{-1}$ (where the conversion has dropped to 27%) it was reduced to 8 min. Conversely, in comparison with dynamic mixing intensified flow reactors, this indicates that mass transfer and interfacial area are probably weakly dependent on flow rate.

### 3.4. Effect of enzyme concentration

In the CPR different enzyme concentrations (0.5, 1, 3, 5, 10 and 15 g $L^{-1}$) of the aqueous phase were tested for 10 mL $min^{-1}$ mobile phase flow rate, 0.032 mol $L^{-1}$ oleic acid feed concentration, at 800 rpm. Results are shown on Fig. 6.

It is seen on Fig. 6 that the conversion increases quasi linearly with enzyme concentration at low enzyme concentrations (0.5 and 1 g $L^{-1}$). At higher enzyme concentration (beyond 3 g $L^{-1}$), this increase tends to level off. This could be attributed to the saturation of all the interface adsorption sites for the enzyme at this concentration level. This behavior has also been reported in the literature [5,16]. Hari Krishna and Karanth [16] considered that the active sites of the excess enzyme molecules are not exposed to the substrates. In the same way, Saktaweewong et al. [5] reported that

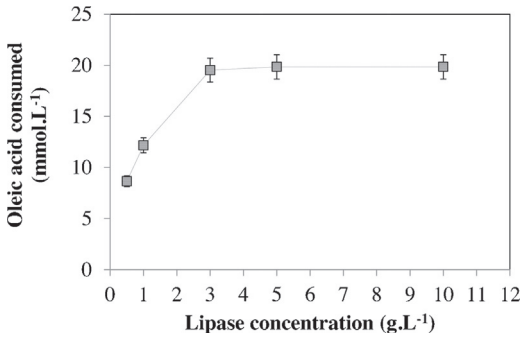


**Fig. 6.** Effect of concentration of the lipase from *R. miehei* upon oleic acid consumed in the CPR, at 800 rpm, *n*-BuOH/oleic acid = 3, oleic acid feed concentration = 0.032 mol $L^{-1}$, and mobile phase flow rate = 10 mL $min^{-1}$. CPR is operated at controlled room temperature (22 °C). The reported conversion values are average of triplicate experiments.

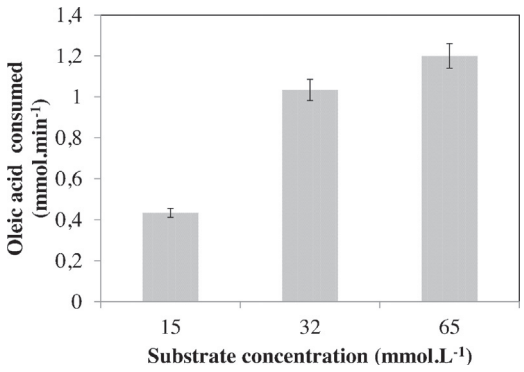


**Fig. 7.** Effect of feed substrate concentration on oleic acid consumed in the CPR. Reactions conditions: 3 g $L^{-1}$ of enzyme, *n*-BuOH/acid ratio of 3, 5 mL $min^{-1}$ flow rate and 800 rpm rotation speed. CPR is operated at controlled room temperature (22 °C).

the lipase activity varied as a function of the ratio of the free available interfacial area to the amount of enzyme. Indeed, excess of lipase at the interface induces a decrease of this ratio, thus the interface becomes saturated and amount of new enzymes able to partition at the interface progressively decreases. Thus, to optimize the conversion, the enzyme concentration corresponding to interface saturation needs to be determined for any given specific surface area.

### 3.5. Effect of substrate concentration

Varying the concentration of the oleic acid in the feed (in the range from 0.016 mol $L^{-1}$ to 0.066 mol $L^{-1}$) has been studied at 3 g $L^{-1}$ enzyme concentration, with *n*-butanol/oleic acid molar ratio of 3, at flow rate of 5 mL $mn^{-1}$ and 800 rpm. Fig. 7 shows the values of the reaction rate defined as the quantity of oleic acid transformed per unit of time. It is seen that the reaction rate increases, (from 0.4 mmol $min^{-1}$ to 1.2 mmol $min^{-1}$) with feed oleic acid concentration, (from 16 mmol $L^{-1}$ to 66 mmol $L^{-1}$). Thus, for the investigated conditions, inhibition by oleic acid is not observed [25–27].

### 3.6. Assessment of the stability and productivity of the batch and CPR reactors

From an industrial point of view it is important to consider the ability of the CPR to insure long time steady operation. So, the CPR was operated during 72 h, and as seen in Fig. 8, an almost constant 85% conversion was observed during the first 24 h, then diminished to 70% after 30 h and to 54% after 72 h. At the end of the experiment,

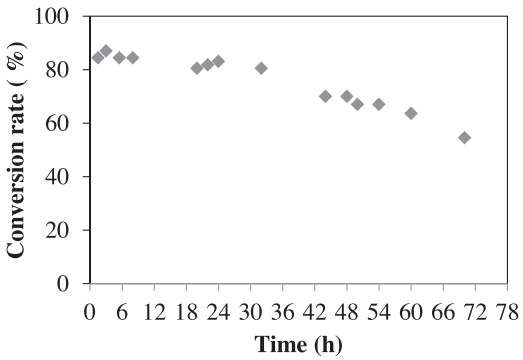


**Fig. 8.** Conversion in the CPR for a 3 day operation. Reaction conditions: 3 g $L^{-1}$ of enzyme, *n*-BuOH/acid ratio of 3, 2.5 mL $min^{-1}$ flow rate and 800 rpm rotation speed. CPR is operated at controlled room temperature (22 °C).

the activity of the lipase used in the CPR was tested using the pNPB test. A decrease from its 917 AU $mL^{-1}$ initial value to 350 AU $mL^{-1}$ was evidenced. In parallel, a lipase solution at 3 g $L^{-1}$ from *R. miehei* was left in the bottle at room temperature during 72 h and a loss of activity of 60% was observed. This indicates that the degradation of the commercial enzyme is the main cause of the diminution of performances of the continuous CPR. Nevertheless, it is possible that part of this loss of stability also originates from shear stresses that the enzyme experiences in the CPR. Another cause could be the occurrence of aqueous phase bleeding during this long duration operation but no significant loss of aqueous phase volume was observed at the end of the experiment.

Another crucial parameter for assessing the CPR as an efficient continuous reactor concerns the productivity of the process. Productivity is the mass of product obtained per unit of time and per unit of utile volume of the reactor and is expressed in g $h^{-1}$ $L^{-1}$. Let us consider a typical case for the CPR: 3 g $L^{-1}$ lipase concentration, 0.032 mol $L^{-1}$ oleic acid feed concentration and a *n*-BuOH/oleic acid molar ratio of 3. Mobile phase flow rate was 10 mL $mn^{-1}$ (residence equal to 8 min) and rotation speed was 800 rpm. With these conditions, conversion was 65% (see Fig 3A). From these values, it can be computed that *n*-butyl oleate was continuously produced with a productivity equal to 40.5 g $h^{-1}$ $L^{-1}$. For the batch process, from the initial slope of the 200 mL experiment (see Fig 3B) a productivity of 21 6 g $h^{-1}$ $L^{-1}$ can be estimated. Therefore, it appears that the productivity of the CPR is comparable and higher than the conventional batch process considered here. In addition to the elimination of inconveniences of batch processing (charge and discharge, quality control...), this result is a good incentive for industrialization of such continuous reactor as an alternative to conventional batch systems.

## 4. Conclusions

This study has demonstrated the potential of the Centrifugal Partition Chromatography apparatus as a novel type of intensified reactor for two phase enzymatic reactions (termed here Centrifugal Partition Reactor, CPR). The esterification of oleic acid by *n*-butanol catalyzed by the *R. miehei* lipase was tested as a model reaction. This enzyme is known to act at the interface and interfacial area and mass transfer properties are therefore prominent parameters. The CPR proved to be efficient to generate high interfacial area even at low rotation speed (800 rpm). As a typical case, steady state operation is reached after about 30 min and 90% esterification conversion can be obtained.

The influence of rotation speed, flow rate, enzyme and substrate concentrations were studied and the obtained results highlighted that, from a kinetic point of view, the esterification reaction performed in the CPR is comparable to the one achieved in the batch reactor that was here operated for comparison purposes.

Nevertheless, when operating the CPR at constant hold-up, the rotation speed surprisingly proved to have a negative effect on conversion, probably related to the specific hydrodynamics inside the CPR cells. So, better understanding of this specific hydrodynamic behavior is still needed to help choosing optimal rotation speed and hold-up in order to optimize the reactor performance. A long duration experiment (72 h) experience showed, after 24 h stability, a diminution of reactor performance that can be mainly attributed to low intrinsic stability of the enzyme at ambient temperature.

For a typical configuration, the productivity of the CPR was evaluated to be more favorable than the conventional batch reactor. In addition, the CPR has other benefits: a liquid–liquid separation for catalyst recycling is not necessary because this step is integrated in the system and also, compared to batch reactor, continuous operation eliminates a lot of manipulations in the process (vessel discharge, introduction of reactants...). This study has illustrated the advantage of the CPC equipment as an intensified reactor for two phase enzymatic reactions and the promising results obtained in this study are a good incentive to promote the CPR as a competitive innovative technology for industrial enzymology. Although centrifuge devices are usually known as high energy consuming devices [28], in the CPC system, conversely to centrifuge contactors, there is no relative movement of the walls. So, maintaining the rotation only requires providing energy to overcome friction for moving metallic pieces, which can be very low for mechanically well designed systems. The main consumption is then the power for pumping which depends on the pressure drop of the mobile phase flow. Rough preliminary calculations (not presented here) enable us to estimate that energy consumption of the CPR is of the same order of magnitude than for a stirred tank reactor of the same volume. Indeed, the CPR equipment does not aim at competing with the batch stirred tank reactor on the point of view of energy consumption. A possible advantage of the stirred tank reactor on this criterion would be largely balanced by the interest of operating a continuous steady production, which is not easily possible in a stirred biphasic batch reactor.

Finally, note that in this study experiments were made using a CPC apparatus in its conventional configuration, which is designed for performing chromatographic separations (very high number of small contact cells). A specific optimized design and operating conditions still have to be proposed to efficiently perform enzymatic reactions (phase ratio, interfacial area, residence time...) using this novel technology.

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